

## Synthesis of Uniform Mesoporous Carbon Capsules by Carbonization of Organosilica Nanospheres

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A synthetic method to produce uniform mesoporous hollow carbon nanospheres with a large surface area and uniform mesoporosity has been developed by employing, as carbon source, an organic moiety used as porogen agent to synthesize spherical organosilica nanoparticles with a core@shell (organic–inorganic) structure. The conversion of the organic moiety to carbon is achieved by means of sulphuric acid which considerably increases the carbon yield via dehydration and sulphonation reactions. The carbon capsules exhibit a uniform morphology (a diameter of  $\sim 440$  nm and a shell thickness of  $\sim 50$  nm), a high Brunauer–Emmett–Teller (BET) surface area ( $1620 \text{ m}^2 \text{ g}^{-1}$ ), a large pore volume ( $2.3 \text{ cm}^3 \text{ g}^{-1}$ ), and a porosity made up of mesopores centered at around 4.3 nm. Iron oxide magnetic nanoparticles were incorporated into the pores of the porous shell of the carbon capsules. The magnetic hollow nanoparticles were then used as support for the immobilization of the cytochrome C. A large amount of enzyme is stored in this magnetic nanocomposite ( $\sim 500 \text{ mg Cyt} \cdot \text{g}^{-1}$  support) which suggests that a significant fraction of enzyme is accommodated in the hollow core of the capsules.

### Introduction

The fabrication of uniform sub-micrometric hollow particles (capsules) with an inorganic (silica, carbon, oxides, etc.) or polymeric framework has recently attracted great attention because these materials have the unique feature of providing a protected macroporous core suitable for encapsulating a large variety of substances.<sup>1</sup> Capsules with a porous shell are of great importance because the permeable layer allows the species to be transported between the macroporous core and the exterior of the capsule and also because the pores in this layer can be used to either incorporate, adsorb, or immobilize guest substances.<sup>2</sup> In particular, sub-micrometric sized hollow carbon particles are of special interest because they combine a high permeability through the porous shell with a good chemical inertness and electronic conductivity. In addition, they are able to incorporate new

functionalities with specific properties (i.e., magnetic, catalytic, electrochemical, etc.) by means of the insertion, into the macroporous core or into the pores of the shell, of inorganic nanoparticles.<sup>3</sup> Such multifunctional hollow carbon particles are very attractive as high-performance catalysts, drug delivery systems, or for the adsorption and immobilization of biomolecules. Several examples of these nanocomposites have been presented recently. Thus, Kim et al. reported the fabrication of Au nanoparticles trapped inside the macroporous core of carbon capsules.<sup>4</sup> Chai et al. described the incorporation of PtRu nanoparticles inside the porous shell of carbon capsules and observed that this material exhibits a high specific activity for methanol electrooxidation.<sup>5</sup> Ikeda et al. demonstrated that platinum nanoparticles encapsulated in a hollow porous carbon shell display a better catalytic performance for hydrogenation reactions than conventional catalysts (i.e., Pt supported on an activated carbon).<sup>6</sup> Recently we reported the fabrication of core@shell materials made up of inorganic nanoparticles confined within the macroporous core of carbon capsules with a mesoporous shell.<sup>7</sup> In addition, we demonstrated the importance of this core@shell architecture for encapsulating nanosized catalysts with a high catalytic performance in

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- (1) (a) Ma, Y.; Qi, L. *J. Colloid Interface Sci.* **2009**, *335*, 1. (b) Lou, X.; Archer, L. A.; Yang, Z. *Adv. Mater.* **2008**, *20*, 3987.
- (2) (a) Yu, A.; Wang, Y.; Barlow, E.; Caruso, F. *Adv. Mater.* **2005**, *17*, 1737. (b) Zhu, Y.; Kockrick, E.; Ikoma, T.; Hanagata, N.; Kaskel, S. *Chem. Mater.* **2009**, *21*, 2547. (c) Zhao, W.; Lang, M.; Li, Y.; Li, L.; Shi, J. *J. Mater. Chem.* **2009**, *19*, 2778. (d) Zhu, Y.; Shi, J.; Shen, W.; Dong, X.; Feng, J.; Ruan, M.; Li, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 5083.
- (3) (a) Kim, M.; Sohn, K.; Na, H. B.; Hyeon, T. *Nano Lett.* **2002**, *2*, 1383. (b) Zhang, W.-M.; Hu, J.-S.; Guo, Y.-G.; Zheng, S.-F.; Zhong, L.-S.; Song, W.-G.; Wan, L.-J. *Adv. Mater.* **2008**, *20*, 1160. (c) Guo, L.; Cui, X.; Li, Y.; He, Q.; Zhang, L.; Bu, W.; Shi, J. *Chem. Asian J.* **2009**, *4*, 1480. (d) Fang, B.; Kim, J. H.; Lee, C.; Yu, J.-S. *J. Phys. Chem. C* **2008**, *112*, 639. (e) Zheng, R.; Meng, X.; Tang, F.; Zhang, L.; Ren, J. *J. Phys. Chem. C* **2009**, *113*, 13065.

- (4) Kim, J. Y.; Yoon, S. B.; Yu, J.-S. *Chem. Commun.* **2003**, 790.
- (5) Chai, G. S.; Yoon, S. B.; Kim, J. H.; Yu, J.-S. *Chem. Commun.* **2004**, 2766.
- (6) Ikeda, S.; Ishino, S.; Harada, T.; Okamoto, N.; Sakata, T.; Mori, H.; Kuwabata, S.; Torimoto, T.; Matsumura, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 7063.
- (7) Fuertes, A. B.; Sevilla, M.; Valdes-Solis, T.; Tartaj, P. *Chem. Mater.* **2007**, *19*, 5418.

heterogeneous processes.<sup>8</sup> A methodology for the preparation of uniform mesoporous carbon capsules with a mesoporous shell (SCMS) was first described by Hyeon et al.<sup>9</sup> They chose a hard-templating route based on using monodisperse sub-micrometric solid core/mesoporous shell (SCMS) silica spheres as sacrificial templates.<sup>10</sup> This type of silica particle is obtained by growing, upon the surface of the Stöber silica spheres, a mesoporous silica shell produced by the co-condensation of tetraethylorthosilicate (TEOS) with a porogen agent such as octadecyltrimethoxysilane (C<sub>18</sub>-TMS). On the other hand, Arnal et al. reported the fabrication of mesoporous hollow carbon microspheres by using as template a mesoporous binary oxide with a core@shell structure (SiO<sub>2</sub>@ZrO<sub>2</sub>).<sup>11</sup>

The conventional synthetic strategy for obtaining mesoporous carbon capsules necessitates several supplementary steps: (i) the infiltration of the mesopores of calcined SCMS silica particles by a carbon precursor (a phenol-formaldehyde mixture, furfuryl alcohol, glucose, etc.), (ii) the polymerization of this precursor, (iii) the carbonization of the polymer, and (iv) the removal of the silica framework. This methodology involves a complex synthetic procedure consisting of multiple steps and the utilization of foreign substances such as carbon precursors and polymerization catalysts. In addition, as Ikeda et al. have emphasized, the infiltration method cannot guarantee the exclusive incorporation of the carbon precursor into mesopores of SCMS, as it also provokes the aggregation of particles.<sup>12</sup> Therefore, the development of simpler synthetic routes toward the formation of uniform porous carbon capsules is still an important challenge. In the present work we report an easy synthetic strategy to obtain uniform and non-aggregated sub-micrometric spherical carbon capsules with a hollow macroporous core and a mesoporous shell structure. The synthetic scheme presented here employs as carbon precursor the organic moiety  $-(\text{CH}_2)_{17}-\text{CH}_3$  of the organosilicon compound (C<sub>18</sub>-TMS) to act as porogen agent in the synthesis of SCMS silica particles. Additionally, we investigate the mechanism of carbon formation, the incorporation of magnetic functionalities into the carbon capsules obtained, and the possibility of using these magnetic hollow particles to immobilize the enzyme cytochrome C. The advantage of the methodology presented here is that it does not require the incorporation of polymeric carbon precursors and that it also avoids the infiltration step, thereby ensuring the formation of isolated hollow particles. Finally, it should be noted that this synthetic strategy opens up a novel route toward the synthesis of nanoporous carbons by employing, as carbon precursors, organic moieties attached to silica in organic-inorganic hybrid materials.

## Experimental Section

**Preparation of the Carbon Capsules.** Sub-micrometer-size solid core/mesoporous shell (SCMS) silica particles were prepared as reported by Unger et al.<sup>10</sup> In a typical synthesis of hollow carbon spheres, 1 g of uncalcined SCMS particles was treated with H<sub>2</sub>O<sub>2</sub> (30 wt % in water) at room temperature for 15 h. The oxidized product was collected by centrifugation and redispersed in a mixture of 10 g of H<sub>2</sub>O and 18 mmol of H<sub>2</sub>SO<sub>4</sub> (H<sub>2</sub>SO<sub>4</sub>/C<sub>18</sub>-TMS molar ratio  $\sim 12$ ). This mixture was heat treated in air at 100 °C for 5 h and at 160 °C for 15 h. The dark solid obtained was heated under N<sub>2</sub> up to 800 °C (3 K/min) for 1 h. Finally, the carbonized sample was treated with hydrofluoric acid (40%) to dissolve the silica framework. The carbonaceous residue was collected by filtration, washed with distilled water, and dried at 120 °C. This type of carbon capsule is designated as HC-C<sub>18</sub>.

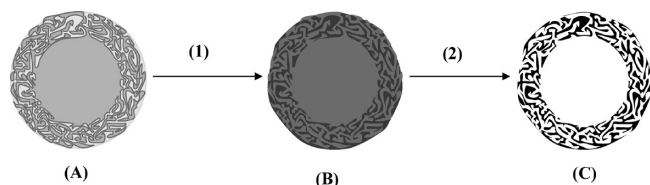
For comparison purposes, carbon capsules with macroporous hollow cores and mesoporous shells were prepared according to the conventional infiltration procedure reported by Hyeon et al.<sup>9</sup> Here we employed paratoluenesulphonic acid as polymerization catalyst and furfuryl alcohol as carbon precursor, which were infiltrated into the mesopores of calcined SCMS silica particles. The mesoporous carbon capsules prepared following this procedure are denoted as HC-FA.

**Incorporation of Magnetic Nanoparticles within the Pores of the Carbon Capsules.** To incorporate the iron oxide ferrite nanoparticles into the hollow carbon particles, the carbon capsules were impregnated with a solution formed by Fe (III) nitrate (Aldrich) dissolved in ethanol ( $\sim 0.2$  g iron nitrate/g ethanol). This solution was added to the carbon particles dropwise until incipient wetness, and then the impregnated sample was dried at 50 °C in a vacuum oven. This process was repeated several times until the desired amount of inorganic precursor was attained ( $\sim 31$  wt % of the inorganic phase in the composite). To convert the inorganic precursor into oxide ferrite nanoparticles, the impregnated sample was exposed, in a closed vessel, to propionic acid vapors at 80 °C for 15 h and then thermally treated under N<sub>2</sub> at 260 °C for 2 h. The resulting composite was denoted as mHC-C<sub>18</sub>.

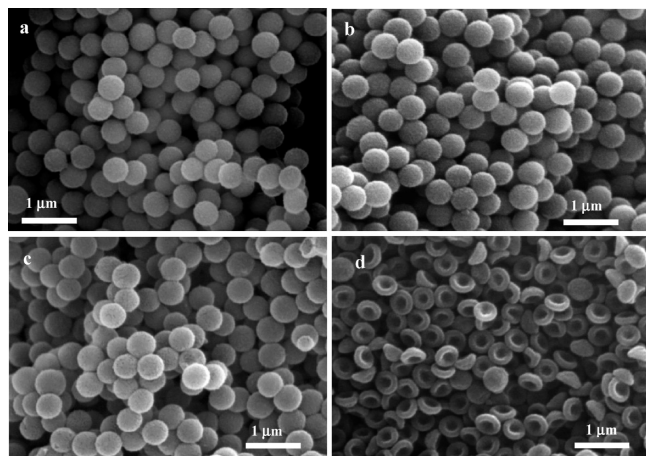
**Materials Characterization.** The morphology of the powders was examined by scanning (SEM, Zeiss DSM 942) and transmission (TEM, JEOL-2000 FXII) electron microscopy. Nitrogen adsorption and desorption isotherms were performed at  $-196$  °C in a Micromeritics ASAP 2020 volumetric adsorption system. The Brunauer–Emmett–Teller (BET) surface area was deduced from an analysis of the isotherm in the relative pressure range of 0.04–0.20. The total pore volume was calculated from the amount of nitrogen adsorbed at a relative pressure of 0.99. The pore size distribution (PSD) was calculated by means of the Kruk–Jaroniec–Sayari method.<sup>13</sup> The primary mesopore volume ( $V_m$ ) and external surface area ( $S_{\text{ext}}$ ) were estimated using the  $\alpha_s$ -plot method. The reference adsorption data used for the  $\alpha_s$  analysis of the silica and carbon samples correspond to a macroporous silica sample<sup>14</sup> and a non-graphitized carbon black sample,<sup>15</sup> respectively. Diffuse reflectance Fourier-Transform Infrared (FT-IR) spectra were recorded on a Nicolet Magna-IR 560 spectrometer fitted with a diffuse reflection attachment. The thermogravimetric analysis was performed in a CI Electronics system. Temperature programmed desorption (TPD) experiments

- (8) Valdés-Solis, T.; Valle-Vigón, P.; Sevilla, M.; Fuertes, A. B. *J. Catal.* **2007**, *251*, 239.  
(9) (a) Bon, S.; Sohn, Y. K.; Kim, J. Y.; Shin, C.-H.; Yu, J.-S.; Hyeon, T. *Adv. Mater.* **2002**, *14*, 19. (b) Kim, M.; Yoon, S. B.; Sohn, K.; Kim, J. Y.; Shin, C.-H.; Hyeon, T.; Yu, J.-S. *Microporous Mesoporous Mater.* **2003**, *63*, 1.  
(10) Büchel, G.; Unger, K. K.; Matsumoto, A.; Tsutsumi, K. *Adv. Mater.* **1998**, *10*, 1036.  
(11) Arnal, P. M.; Schuth, F.; Kleitz, F. *Chem. Commun.* **2006**, 1203.  
(12) Ikeda, S.; Tachi, K.; Ng, Y.; Ikoma, Y.; Sakata, T.; Mori, H.; Harada, T.; Matsumura, M. *Chem. Mater.* **2007**, *19*, 4335.

- (13) Kruk, M.; Jaroniec, M.; Sayari, A. *Langmuir* **1997**, *13*, 6267.  
(14) Jaroniec, M.; Kruk, M.; Oliver, J. P. *Langmuir* **1999**, *15*, 5410.  
(15) Kruk, M.; Jaroniec, M.; Gadkaree, K. P. *J. Colloid Interface Sci.* **1997**, *192*, 250.



**Figure 1.** Illustration of the synthesis procedure. (A) Organosilica nanospheres ( $S-C_{18}$ ); (B) Silica/Carbon composite ( $S-C$ ); (C) mesoporous carbon capsule ( $HC-C_{18}$ ). (1) Oxidation-sulphonation-carbonization of the organic moiety of  $C_{18}$ -TMS; (2) etching of silica.



**Figure 2.** SEM images of (a) solid core mesoporous shell silica particles, (b) silica-carbon nanospheres, (c) spherical mesoporous carbon capsules, and (d) deflated mesoporous carbon capsules.

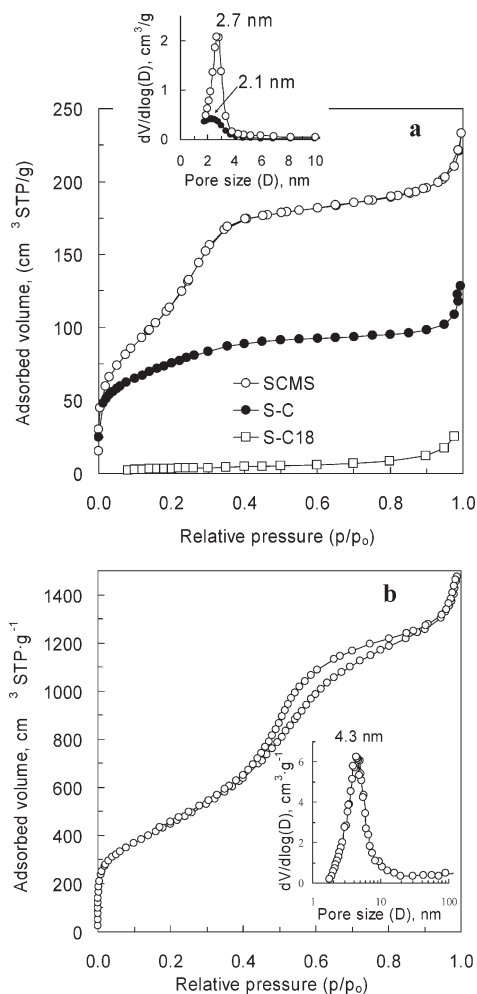
were carried out in a chemisorption analyzer (Micromeritics, Autochem II) by heating the samples up to 800 °C in a  $N_2$  flow at a rate of 10 °C/min and recording the amounts of released gases on a mass spectrometer (OmniStar 3000). The Raman spectra were recorded on a Horiva (LabRam HR-800) spectrometer. The source of radiation was a laser operating at a wavelength of 514 nm and a power of 25 mW. X-ray photoelectron spectroscopy (XPS) was performed on a Specs spectrometer, using  $Mg\ K\alpha$  (1253.6 eV) radiation from a double anode at 50 W. X-ray diffraction (XRD) patterns were obtained on a Siemens D5000 instrument operating at 40 kV and 20 mA and using  $Cu\ K\alpha$  radiation ( $\lambda = 0.15406$  nm).

**Immobilization of Cytochrome C over Carbon Capsules.** For cytochrome C adsorption experiments, a series of standard solutions with concentrations in the 23–230  $\mu M$  range were prepared by dissolving cytochrome C in a 25 mM stock buffer solution (Borax/NaOH buffer, pH 9.3). Around 10 mg of support was dispersed in 10 mL of the enzyme solution. The mixture was stored in a closed vessel and stirred at room temperature by using an orbital stirrer for around 60 h. To determine the amount of enzyme immobilized, the concentration in the solution was monitored by means of a UV-vis spectrophotometer (Shimadzu UV-2401PC) at a wavelength of 409 nm.

## Results and Discussion

### Morphology and Structural Properties of Materials.

The synthetic scheme used to obtain these mesoporous carbon capsules is illustrated in Figure 1. The structural characteristics of the organosilica nanospheres ( $S-C_{18}$ ), the silica-carbon composite ( $S-C$ ), the calcined silica (SCMS), and the hollow carbon particles ( $HC-C_{18}$ ) were



**Figure 3.** Nitrogen sorption isotherm and pore size distributions (Insets) of (a) silica (SCMS) and silica composites ( $S-C_{18}$  and  $S-C$ ), and (b) carbon capsules ( $HC-C_{18}$ ).

investigated by means of SEM, TEM, and nitrogen physisorption. The SEM images for these samples are shown in Figure 2. It can be seen that the external appearance of the nanospheres of all the samples are identical and that they have a very uniform diameter of around  $440 \pm 20$  nm. The as-prepared sample ( $S-C_{18}$ ) that contains the organic  $-(CH_2)_{17}-CH_3$  group attached to the silica is nonporous, as can be deduced from the nitrogen sorption isotherm shown in Figure 3a ( $S_{BET} < 15\ m^2\ g^{-1}$ ). This proves that the organic moiety is thoroughly incorporated into the shell layer of the organosilica nanospheres. When the  $S-C_{18}$  sample is calcined, the resulting silica sample (SCMS) exhibits a well-developed porosity, generated as consequence of the removal of the organic group. Thus, it exhibits a BET surface area of  $450\ m^2\ g^{-1}$  and a porosity made up of uniform mesopores centered at 2.7 nm, as can be seen from the pore size distribution (see inset in Figure 3a). The textural properties of these materials are summarized in Table 1. The treatment of the  $S-C_{18}$  composite with sulphuric acid and the subsequent heat treatment following the methodology described in the Experimental Section leads to the formation of a silica-carbon composite ( $S-C$ ), with a certain porosity (see Figure 3a and Table 1). This porosity consists



**Table 1. Textural Properties of the Organosilica (S-C<sub>18</sub>), Silica (SCMS), Silica-Carbon Composite (S-C), and Hollow Carbon (HC) Particles**

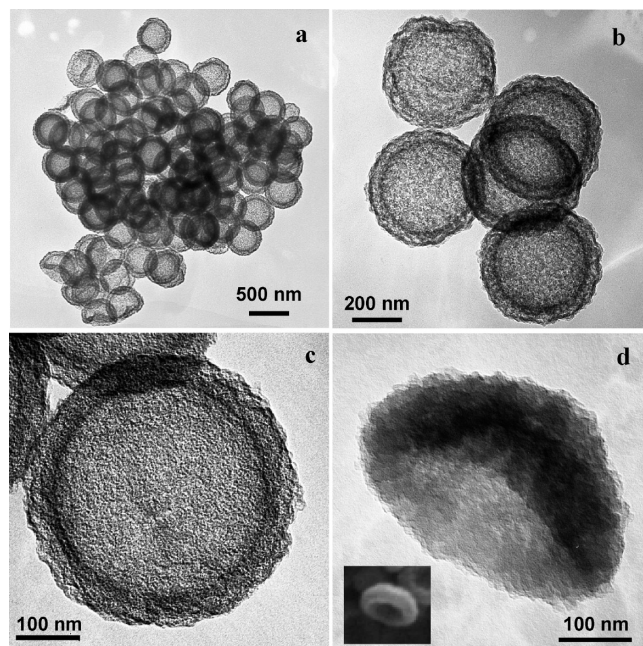
sample	$S_{\text{BET}}$ ( $\text{m}^2 \text{g}^{-1}$ )	$V_{\text{p}}$ ( $\text{cm}^3 \text{g}^{-1}$ ) <sup>a</sup>	pore size (nm) <sup>b</sup>	fwhm (nm) <sup>c</sup>	$\alpha_s$ -plot results	
					$V_{\text{m}}$ ( $\text{cm}^3 \text{g}^{-1}$ ) <sup>d</sup>	$S_{\text{ext}}$ ( $\text{m}^2 \text{g}^{-1}$ ) <sup>e</sup>
S-C <sub>18</sub>	14	<0.05				
SCMS	450	0.36	2.7	0.9	0.24	58
S-C	270	0.2	2.1		0.13	14
HC-C <sub>18</sub>	1620	2.3	4.3	2.8	1.73	168
HC-FA	2040	2.1	3.5	1.8	1.71	113

<sup>a</sup> Pore volume determined at  $p/p_0 = 0.99$ . <sup>b</sup> Maximum of the pore size distribution. <sup>c</sup> Full Width at Half Maximum of the pore size distribution. <sup>d</sup> Pore volume of structural mesopores. <sup>e</sup> External surface area.

of pores of around 2 nm (see inset in Figure 3a), which are generated by the release of volatile gases produced during pyrolysis. From a comparison of the textural properties of the SCMS and the S-C samples it can be deduced that the carbon present in the S-C composite occupies  $\sim 50\%$  of the pore volume of the SCMS silica.

The dissolution of the silica framework of the silica-carbon particles (S-C) gives rise to uniform carbon nanospheres with a diameter of around 440 nm, as can be seen from the SEM image (see Figure 2c). These particles exhibit hollow cores as the TEM images in Figure 4a–c clearly show. The diameter of the macroporous core is around 350 nm. The shell of these capsules, which has a thickness of  $\sim 50$  nm, contains framework-confined pores, as revealed by the TEM image shown in Figure 4c. The textural characteristics of these porous capsules were examined by means of the nitrogen sorption isotherm and pore size distribution (Figure 3b). There is a large nitrogen uptake for  $p/p_0 > 0.9$ , indicating a textural porosity that is related to the presence of interparticle voids between the hollow particles. The carbon capsules have a large BET surface area of  $1620 \text{ m}^2 \text{g}^{-1}$  and a high pore volume of  $2.3 \text{ cm}^3 \text{g}^{-1}$  ( $1.73 \text{ cm}^3 \text{g}^{-1}$  corresponding to the shell-confined pores, as can be deduced from the  $\alpha_s$ -plot analysis of the adsorption branch). Moreover, the porosity is made up of mesopores in the  $\sim 2$ – $10$  nm range (full width at half-maximum, fwhm, of 2.8 nm) the maximum being centered at 4.3 nm (see Figure 3b, inset). These results clearly show the successful formation of mesoporous carbon capsules following the methodology presented.

The morphology of the carbon particles largely depends on the carbon yield ( $X_C$ ) which is here defined as the weight fraction of the alkyl group  $-(\text{CH}_2)_{17}-\text{CH}_3$  that is converted to carbon. Thus, for  $X_C > 0.25$ , the synthesized hollow carbon nanoparticles have a spherical morphology (capsules), whereas for  $X_C < 0.25$ , the particles appear as deflated capsules with a folded porous carbon shell. This suggests that in the latter case, the carbon shell lacks the mechanical strength needed to maintain its spherical shape. We have investigated the effects of a variety of synthetic parameters on the carbon yield and found that it mainly depends on the ( $\text{H}_2\text{SO}_4/\text{C}_{18}\text{-TMS}$ ) molar ratio employed for the synthesis. Thus, for ( $\text{H}_2\text{SO}_4/\text{C}_{18}\text{-TMS}$ )  $< 8$ , the result is  $X_C < 0.25$ , and, consequently, the carbon particles produced appear as

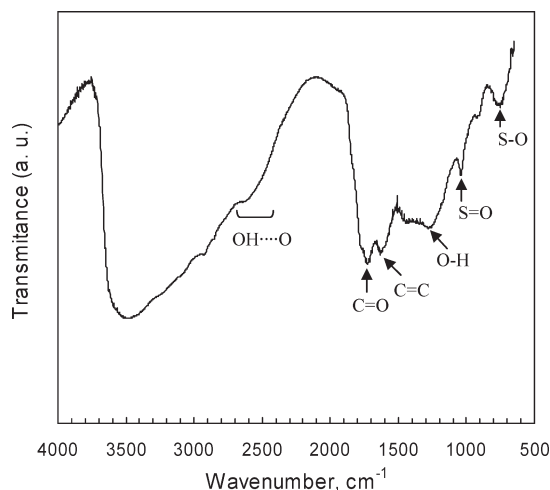


**Figure 4.** TEM images of hollow mesoporous carbon nanospheres (a, b, and c) and deflated carbon capsules (d). Inset in (d) shows a SEM image of a deflated capsule.

deflated capsules. The morphology of these deflated capsules is illustrated by the SEM (Figure 2d) and TEM (Figure 4d) images. In our procedure we selected a molar ratio ( $\text{H}_2\text{SO}_4/\text{C}_{18}\text{-TMS}$ ) = 12, which we consider to be the optimal for obtaining carbon capsules with a spherical morphology. The modification of the appearance of the hollow carbon nanoparticles from spherical to deflated does not imply a substantial modification in the textural properties, as can be seen from a comparison of the nitrogen sorption isotherms and pore size distributions obtained for both types of carbon nanoparticles (see Supporting Information, Figure S1).

A comparison of the structural (TEM images) and textural (nitrogen sorption isotherms and pore size distributions) properties of the carbon capsules prepared according to the methodology reported in the present work (HC-C<sub>18</sub>) with those synthesized by a conventional impregnation method (HC-FA) is shown in the Supporting Information, Figure S2. It reveals that, whereas the structural properties (i.e., diameter of the nanospheres and thickness of the porous shell) are analogous in both cases, the textural characteristics are somewhat different (see Table 1). The most important difference is related to pore size, which is larger in the HC-C<sub>18</sub> capsules than in the HC-FA ones.

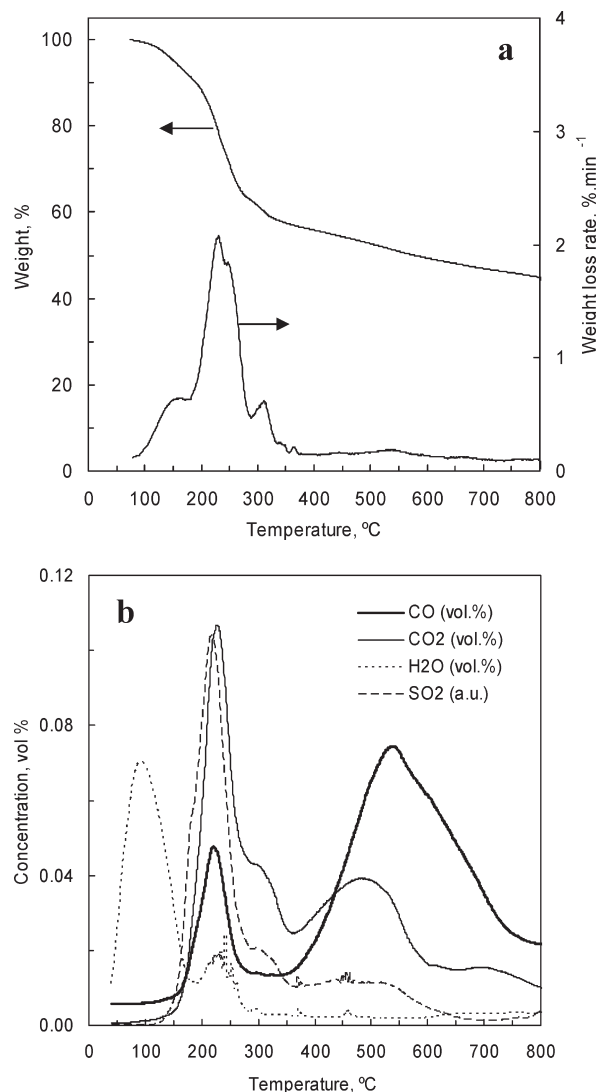
**Mechanism of the Formation of the Carbonaceous Material.** The conversion of the alkyl group  $-(\text{CH}_2)_{17}-\text{CH}_3$  attached to silica to carbon was investigated. In the absence of sulphuric acid, this organic group completely decomposes into gaseous species during pyrolysis (see the Supporting Information, Figure S3). In contrast, when the process occurs in the presence of sulphuric acid, a substantial fraction of the organic moiety is converted into carbon (up to 30 wt %). The conversion of the organic



**Figure 5.** FTIR spectrum of the S-C<sub>18</sub> composite treated with H<sub>2</sub>SO<sub>4</sub> at 160 °C for 15 h.

substances into carbon in the presence of sulphuric acid is a procedure commonly employed to fabricate templated mesoporous carbons.<sup>16</sup> Thus, Kim et al. reported the fabrication of mesoporous carbons by means of the carbonization of the surfactant (Pluronic P123) confined inside the porosity of the silica, with the aid of the sulphuric acid.<sup>17</sup> Moreover, several authors have found that the sulphonation of polymeric resins increases the carbonization yield.<sup>18</sup> However, although the sulphuric acid has been widely employed to prepare carbon materials, its role in the conversion of organic molecules to carbon has hardly been investigated.

In a first step the organosilica microspheres were oxidized with H<sub>2</sub>O<sub>2</sub>. We observed that this pre-treatment causes a slight increment of the carbon yield (~10 wt %), which is important to obtain robust carbon capsules. In our opinion, this is a consequence of the fact that the oxidation of the C<sub>18</sub>-alkyl chain by H<sub>2</sub>O<sub>2</sub> introduces oxygen functionalities, which favors the dehydration reactions by the H<sub>2</sub>SO<sub>4</sub>. To clarify the role of the sulphuric acid in the formation of the carbonaceous material, we analyzed the chemical characteristics of composites at several stages during carbonization. Thus, for a composite obtained after treatment with sulphuric acid at 160 °C and subsequently washed with water to remove the unreacted sulphuric acid, the FTIR spectrum (Figure 5) clearly reveals the formation of sulfonic groups (–SO<sub>3</sub>H). Indeed, the band at 780 cm<sup>–1</sup> can be assigned to S–O stretching vibrations, whereas the sharp peak at 1050 cm<sup>–1</sup> is associated to symmetric S=O stretching vibrations.<sup>19</sup> Important also is the presence of a broad band at 2450–2700 cm<sup>–1</sup>, which can be assigned to an overtone of the

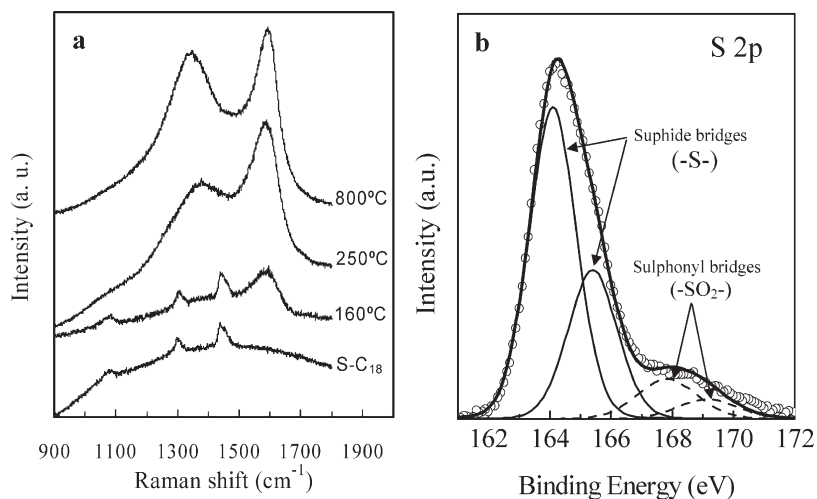


**Figure 6.** (a) Modification of the weight and weight loss rate (TGA experiment), and (b) the concentration of evolved gases (TPD experiment) during the thermal treatment under nitrogen of a sample treated in air at 160 °C (15 h) in the presence of H<sub>2</sub>SO<sub>4</sub>. Heating rate: 5 °C/min.

bending mode of OH · · · O linked by a strong hydrogen bond, suggesting that some –SO<sub>3</sub>H groups are contiguous.<sup>20</sup> The other bands that appear in the spectrum can be assigned to C=O stretching (1730 cm<sup>–1</sup>), C=C stretching (1630 cm<sup>–1</sup>), CH<sub>3</sub> asymmetric bending (1450 cm<sup>–1</sup>), and O–H bending (1300 cm<sup>–1</sup>). The presence of C=C linkages suggests that in parallel with the sulphonation of the organic moiety, a dehydration process occurs. The heat treatment of the sulphonated sample causes the sulfonic groups to decompose, as evidenced by the results obtained by the thermogravimetric analysis (TGA) and the temperature-programmed desorption (TPD) experiments (Figure 6). The weight of the sample undergoes a pronounced decrease in the 150–350 °C range, maximum rate of weight loss occurring at around 230 °C (Figure 6a). This weight loss can be ascribed to the decomposition of the unreacted sulphuric acid (H<sub>2</sub>SO<sub>4</sub> → H<sub>2</sub>O + SO<sub>3</sub>) and of a substantial fraction of the previously generated sulfonic

- (16) (a) Ryoo, R.; Joo, S.-H.; Jun, S. *J. Phys. Chem. B* **1999**, *103*, 7743. (b) Jun, S.; Joo, S. H.; Ryoo, R.; Kruk, M.; Jaroniec, M.; Liu, Z.; Ohsuna, T.; Terasaki, O. *J. Am. Chem. Soc.* **2000**, *122*, 10712.  
(17) Kim, J.; Lee, J.; Hyeon, T. *Carbon* **2004**, *42*, 2711.  
(18) (a) Neely, J. W. *Carbon* **1981**, *19*, 27. (b) Matsuda, M.; Funabashi, K. *J. Polym. Sci., Part A: Polym. Chem.* **1987**, *25*, 669. (c) Malika, D. J.; Trochimczuk, A. W.; Jyoc, A.; Tylusd, W. *Carbon* **2008**, *46*, 310.  
(19) (a) Sahin, Y.; Aydin, A.; Udum, Y. A.; Pekmez, K.; Yıldız, A. *J. Appl. Polym. Sci.* **2004**, *93*, 526. (b) Tian, X.; Su, F.; Zhao, X. S. *Green Chem.* **2008**, *10*, 951.

- (20) Suganuma, S.; Nakajima, K.; Kitano, M.; Yamaguchi, D.; Kato, H.; Hayashi, S.; Hara, M. *J. Am. Chem. Soc.* **2008**, *130*, 12787.



**Figure 7.** (a) Raman spectra of the samples collected at different stages of the carbonization process. (b) S<sub>2p</sub> XPS spectrum of a silica–carbon composite carbonized at 800 °C under N<sub>2</sub>.

groups, as can be deduced from the TPD (Figure 6b), which shows that the release of SO<sub>2</sub>, H<sub>2</sub>O, CO, and CO<sub>2</sub> reaches a maximum at 220–230 °C. The peaks of the CO and CO<sub>2</sub> released at this temperature reveal that, as the sulphuric acid and the sulfonic groups decompose, the carbonaceous material is partially gasified because of its oxidation by the in situ produced SO<sub>3</sub> (SO<sub>3</sub> + C → SO<sub>2</sub> + CO/CO<sub>2</sub>). More detailed information about the evolution of the chemical nature of the carbonaceous material with temperature can be deduced from an analysis of the Raman spectra obtained for the samples at several stages of carbonization. The as-synthesized organosilica composite S–C<sub>18</sub> exhibits three peaks which are characteristic of the saturated alkyl chain associated to silica (≡Si–(CH<sub>2</sub>)<sub>17</sub>–CH<sub>3</sub>).<sup>21</sup> The sample treated with sulphuric acid at 160 °C exhibits an additional weak band centered at 1580 cm<sup>-1</sup>, which is characteristic of alkene stretching vibrations<sup>21</sup> and suggests the formation of C=C linkages because of dehydration reactions catalyzed by the sulphuric acid. This conclusion is coherent with what was previously deduced from the FTIR analysis (see Figure 5). The composite obtained by heat treatment up to 250 °C exhibits two broad overlapping bands at around 1360 (D-mode) and 1587 (G-mode) cm<sup>-1</sup>, which reveal the presence of C sp<sup>2</sup> atoms in benzene or condensed benzene rings of amorphous (partially hydrogenated) carbon<sup>22</sup> and aromatic carbon clusters. Finally, it can be seen that the G-band in the sample carbonized at 800 °C is narrower than the one at 250 °C, indicating an increase in the size of the aromatic clusters in the carbonaceous material. This result is corroborated by the XRD pattern corresponding to the carbon capsules obtained at 800 °C (see the Supporting Information, Figure S4). This pattern exhibits a well-defined broad (002) diffraction peak (2θ ~ 10–30°) attributable to amorphous carbon composed of aromatic carbon sheets oriented in a considerably random fashion.

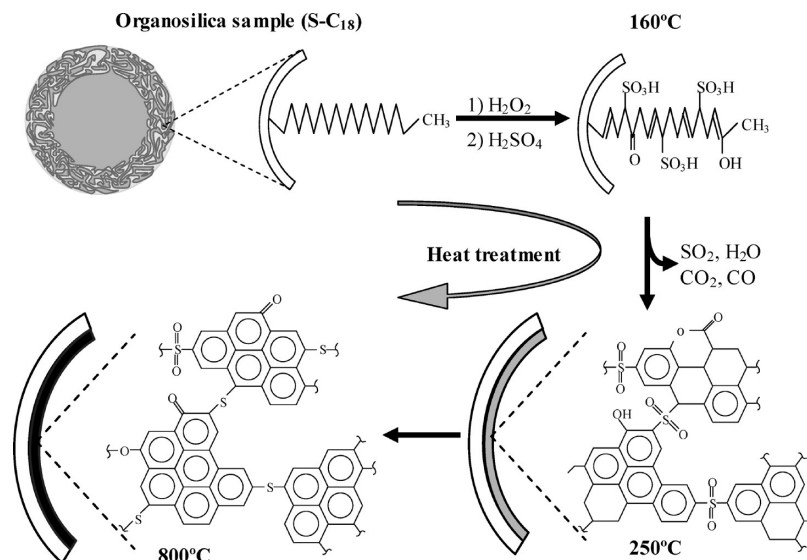
The sulfur content associated to the carbonized sample (800 °C) was of the order of ~1.5 wt % as determined by elemental analysis. The sulfur functionalities in this sample were examined by means of X-ray photoelectron spectroscopy (XPS). The analysis of the S 2p XPS spectrum (Figure 7b) shows that the sulfur is associated to carbon forming sulphide bridges (–S–, peaks at 164.1 and 165.4 eV) and sulphonyl bridges (–SO<sub>2</sub>–, peaks at 167.8 and 169.1 eV).<sup>18b,c</sup> Most of the sulphonyl bridges formed through the dehydration of sulfonic groups decompose during pyrolysis (via deoxidation reactions) and are transformed into sulphide bridges. In consequence, the sample carbonized at 800 °C has a high (–S–)/(–SO<sub>2</sub>–) ratio of ~6.3 as estimated by XPS analysis. The above results show that sulphuric acid is involved in two types of processes, that is, dehydration reactions and the formation of sulfonic groups, both of which play an important role in the formation of carbonaceous residue. Thus, whereas dehydration reactions favor aromatization, sulphonation increases the carbonization yield because of cross-linking processes that occur through the formation of sulphonyl and sulphide bridges between adjacent phenyl rings. On the basis of the previous results, Figure 8 shows a schematic illustration of the formation of carbonaceous material via the sulphonation of organic alkyl groups attached to silica. We hypothesize that the role of sulphuric acid in the formation of carbon from sucrose, commonly employed to fabricate templated carbons, is similar to that described here in relation to the –(CH<sub>2</sub>)<sub>17</sub>–CH<sub>3</sub> alkyl group.

**Cytochrome C Immobilization over Carbon Capsules with Magnetic Functionalities.** The incorporation of magnetic nanoparticles inside the pores of carbon substrates is especially relevant to applications that require the recycling or the separation of the support from the rest of system. We have applied this concept to carbon capsules fabricated according to the procedure presented before. In a first step, we deposited iron oxide magnetic nanoparticles inside the pores of the porous carbon shell. Then, to demonstrate the applicability of this magnetic

(21) Socrates, G. *Infrared and Raman Characteristic Group Frequencies*, 3rd ed.; Wiley: New York, 2005.

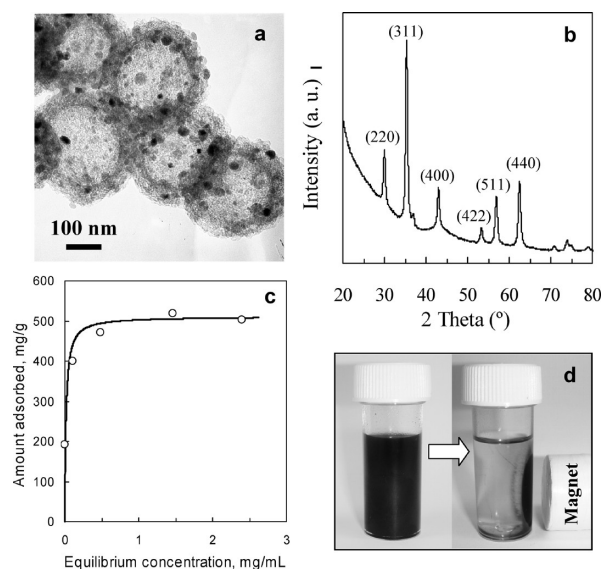
(22) (a) Ferrari, A. C.; Robertson, J. *Phys. Rev. B* **2000**, *61*, 14095. (b) Schwan, J.; Ulrico, S.; Batori, V.; Ehrhardt, H. *J. Appl. Phys.* **1996**, *80*, 440.





**Figure 8.** Schematic illustration of the transformation of the  $-(\text{CH}_2)_{17}-\text{CH}_3$  alkyl group to carbon with the aid of sulphuric acid.

system, we employed the magnetic carbon capsules as support to immobilize an enzyme. The insertion of the magnetic nanoparticles was carried out following the methodology described by us elsewhere.<sup>23</sup> The magnetic carbon capsules (denoted as mHC-C<sub>18</sub>) contained ~31 wt % of iron oxide ferrite nanoparticles, and they have a BET surface area of  $710 \text{ m}^2 \text{ g}^{-1}$ , a framework-confined mesopore volume of  $0.54 \text{ cm}^3 \text{ g}^{-1}$ , and a mesopore size centered at ~3 nm. As a model enzyme for immobilization, we used cytochrome C (pI ~ 9.8, MW = 12.4 kDa, size:  $2.6 \times 3.2 \times 3.3 \text{ nm}^3$ ).<sup>24</sup> The TEM image in Figure 9a reveals that the iron oxide nanoparticles are mainly deposited on the porous shell of the carbon capsules. They consist of iron oxide spinel nanomagnets (magnetite@maghemite) as revealed by the XRD pattern shown in Figure 9b, and they have a size of around 11.5 nm as deduced by applying the Scherrer equation to the (311) diffraction peak. The adsorption isotherm corresponding to the adsorption of cytochrome C by the magnetic hollow particles at pH 9.3 is shown in Figure 9c. It can be seen that these hollow nanoparticles immobilize a large amount of enzyme, the maximum amount being ~500 mg Cyt  $\text{g}^{-1}$ . This quantity is considerably higher than the values found in the literature for conventional mesoporous carbons, in spite of the fact that the magnetic carbon capsules exhibit a lower pore volume. Thus, Vinu et al. reported for CMK-3 templated mesoporous carbons (pore volume:  $1.3 \text{ cm}^3 \text{ g}^{-1}$ ; pore size: ~4.3 nm), a maximum loading of cytochrome C of ~220 mg Cyt  $\text{g}^{-1}$ .<sup>25</sup> This substantial difference in the amounts of enzyme immobilized may be because a significant fraction of the enzyme immobilized is accommodated inside the macroporous core of the carbon capsules. This hollow core acts as a nano-



**Figure 9.** (a) TEM image of the carbon capsules containing magnetic iron oxide nanoparticles (mHC-C<sub>18</sub> sample). (b) XRD pattern of the mHC-C<sub>18</sub> sample. (c) Adsorption isotherm of Cytochrome C on the magnetic carbon capsules (room temperature, pH = 9.3). (d) Example of magnetic separation of the mHC-C<sub>18</sub> capsules loaded with cytochrome C.

container, which considerably enhances the capacity of immobilization in relation to what might be expected from the pore volume of the mesopores in the shell ( $0.54 \text{ cm}^3 \text{ g}^{-1}$ ). An important advantage of using magnetic carbon capsules to immobilize biomolecules is that they can be easily recovered from the reaction media by means of an external magnetic field. Indeed, it has been experimentally observed that enzyme loaded mHC-C<sub>18</sub> particles dispersed in a buffer solution are attracted by a conventional magnet placed close to the vessel, a clear demonstration of the efficacy of magnetic separation (see Figure 9d).

## Conclusions

In summary, we have reported a synthetic procedure for successfully fabricating uniform carbon capsules with a

- (23) (a) Fuertes, A. B.; Valdes-Solis, T.; Sevilla, M.; Tartaj, P. *J. Phys. Chem. C* **2008**, *112*, 3648. (b) Sevilla, M.; Valle-Vigón, P.; Tartaj, P.; Fuertes, A. B. *Carbon* **2009**, *47*, 2519.
- (24) (a) Ochi, H.; Hata, Y.; Tanaka, N.; Kakudo, M.; Sakurai, T.; Aihara, S.; Morita, Y. *J. Mol. Biol.* **1983**, *166*, 407. (b) Macdonald, I. D. G.; Smith, W. E. *Langmuir* **1996**, *12*, 706.
- (25) Vinu, A.; Streb, C.; Murugesan, V.; Hartmann, M. *J. Phys. Chem. B* **2003**, *107*, 8297.

structure consisting of a hollow macroporous core and a mesoporous shell. The procedure employs, as carbon source, an organic moiety that acts as porogen agent in the synthesis of solid core@mesoporous shell silica nanospheres. The conversion of the organic moiety to carbon is achieved by means of sulphuric acid which considerably increases the carbon yield via dehydration and sulphonation reactions. These favor the aromatization and cross-linking processes through the formation of sulphonyl and sulphide bridges. The present approach provides an easy route for synthesizing uniform mesoporous hollow carbon particles. These mesoporous carbon capsules can be used in various applications such as drug delivery or enzyme immobilization. In the present work, we have demonstrated this concept by attaching iron oxide magnetic nanoparticles to the carbon capsules and employing this kind of magnetic

nanocomposite for the immobilization of an enzyme (cytochrome C). The results obtained indicate that these magnetic capsules are able to immobilize a large amount of enzyme and suggest that a considerable fraction of the enzyme is stored in the hollow core of the magnetic capsules.

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**Supporting Information Available:** Additional information in the form of Figures S1–S4. This material is available free of charge via the Internet at <http://pubs.acs.org>.